THE VALUE OF URINARY EXAMINATIONS IN THE DIAGNOSIS OF MALARIA

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INTRODUCTION

The work which follows was commenced in England and continued in West Africa. It was undertaken with the object of ascertaining whether the more commonly employed urinary tests and examinations have any value as aids in the diagnosis of malaria; if they have then their importance is obvious, more especially in those only too frequent cases in which the blood is negative and the patient gives a history of having had ' fever ' a few days previously, for which he took quinine. The urines were examined for urobilinogen, urobilin, albumin, casts, indican, bile pigments, and urea percentage.

Urobilinogen and Urobilin. Considerable difference of opinion exists regarding the importance attachable to the presence of these pigments in the urine. Plehn (1908) believes urobilinuria to be a point of great diagnostic value, but Surveyor (1910), while working in India, was unable to confirm Plehn's observations. Acton and Knowles (1913) state

'Urobilin is not found in the urine of healthy persons, but is found in various fevers, *e.g.*, pneumonia, and the late stages of typhoid and Malta fevers, etc.'

In their summary they state

'The presence of urobilin in the urine in large quantities indicates that haemoglobin is being destroyed. The blood destruction, except in certain well-defined diseases, is due to the destruction of erythrocytes by malarial parasites. If there is no fever present the patient is in the later stages of the disease.'

Acton and Knowles made no quantitative tests and, judging by the context, the expression 'large quantities' simply means a well-marked fluorescence with Schlesinger's solution. Simpson (1910) examined twenty-two cases of malaria and estimated the urinary urobilin quantitatively on several occasions in each individual; he found that in simple tertian malaria the output of urobilin is never greater than in other diseases such as pneumonia. He proceeds :

'In malignant tertian malaria (*P. falciparum*) a different picture is obtained; definite urobilinuria sets in very shortly after the onset of the pyrexia, reaches its height on the second or third day and then slowly diminishes; the normal level of excretion is reached in ten days unless a fresh paroxysm intervenes. Sometimes the increased output continues for a longer period (*e.g.*, Case 20) and in old standing cases with chronic malarial anaemia there may be a continuous raised urobilin output even in the absence of pyrexia (Case 19).'

On looking up Case 20, we find that on seven out of twenty-eight examinations the urobilin output is marked as 'o,' and in Case 19, referred to in the text as ' Continuous raised urobilin output,' three out of eighteen examinations are marked 'o.' Ballerstedt (1924) states in his summary that urobilin persists in the urine of convalescent malarias and for a long time after the cessation of all attacks, but is careful to point out that slight urobilinuria may be found in healthy people; he notes that when the patient's temperature has been normal for some time the urobilinogen test is usually negative while the urobilin remains positive. Sorensen (1914) does not believe in the persistence of urobilin except for two to four days after the subsidence of fever, ' though its reappearance invariably presages a relapse.' Di Pace (1923) after the examination of some one hundred and eighty malarial urines reaches the following conclusions regarding urobilinuria: (I) It is present in all acute malarias with parasites and symptoms still present. (2) Frequent in patients who had an attack of malaria two to three months previously and in whom parasites are still present. (3) Rare in cases with a history of malaria more than three months previously clinically cured, but who show parasites after the administration of strychnine. (4) Extremely rare in latent malarias that are absolutely without symptoms. Di Pace unfortunately appears to have used no controls.

Albumin and casts. Nephritis as a concomitant or complication of malaria is referred to by almost all writers on malaria. Levy Moise (1923) in a paper reviewing previous workers' figures, points out its great frequency. Moise examined seventy-six cases of malaria and found albumin in 27.6 per cent. and casts in 25 per cent. Gordon (1923a) found nephritis in ten out of sixteen cases of malaria examined in England.

Indican, bile pigments, and percentage of urea. These will be dealt with later.

TESTS EMPLOYED IN THE URINARY EXAMINATIONS

All urines were examined as soon after passing as possible and in hospital cases the first urine passed in the morning was always tested.

Urobilinogen. Two drops of Ehrlich's aldehyde reagent, which consists of a 3 per cent. solution of paradimethylaminoazobenzaldehyde in 50 per cent. hydrochloric acid, were added to five c.cs. of urine and the tube gently warmed to blood heat; the appearance of a rose pink to scarlet colour was regarded as positive. MacCormac and Dodds (1923) when describing this reaction state 'This test when positive demonstrates the presence of a pathological amount of urobilinogen.'

In order to prevent the conversion of urobilinogen to urobilin the urine to be tested was exposed to light as little as possible; Graham (1911) in a paper on the effects of quinine on the excretion of urinary pigments, states that he placed ten hour samples of urine in a well-lighted window for two hours in order to 'Ensure the complete conversion of urobilinogen to urobilin.' In connection with this it may be remarked that the present writer frequently noted that a urine giving a positive urobilinogen reaction was still positive after eight hours exposure to full daylight.

Urobilin. Schlesinger's test was used, twenty c.cs. of urine were acidulated with acetic acid and the urobilin extracted by gently inverting with five c.cs. of amyl alcohol. (The mixture must be made gently or an emulsion will result.) The amyl alcohol was then pipetted off and half a c.c. of a 10 per cent. solution of zinc chloride in absolute alcohol added to it; the appearance of distinct fluorescence and a zinc-urobilin band in the spectrum were taken as positive; the spectroscopic result is important, as Ballerstedt (1924) has pointed out that the blue fluorescence of quinine in the urine may influence the typical green fluorescence of urobilin.

Albumin. Sulphosalicylic acid was prepared by dissolving thirteen grammes of salicylic acid in twenty grammes of sulphuric acid, by warming, and after cooling, adding sixty-seven c.cs. of water (Cole (1920)). If no ring resulted on the addition of urine, albumin was regarded as absent; it was found, especially amongst West African natives, that mucin was frequently present and gave a ring indistinguishable from albumin and it was found necessary to exclude this by testing for increased opacity on the addition of dilute acetic acid.

Indican. To five c.cs. of urine in a test-tube were added one large drop of 5 per cent. potassium chlorate, then five c.cs. of strong hydrochloric acid, followed by five c.cs. of chloroform, the contents being mixed by inverting the closed test-tube a couple of times. Definite blue colouration in the separated chloroform was regarded as positive.

Bile pigments. Various reagents were tried and Fouchet's test, as described by MacCormac and Dodd (1923) for testing serum, was found the most satisfactory; to a small quantity of urine was added an equal quantity of the following reagent, trichloracetic acid five grammes, water twenty c.cs. and 10 per cent. ferric chloride solution two c.cs.; bile pigments were indicated by the appearance of a bright green precipitate.

Urea percentage. This was roughly estimated in the usual way with a 'Southall ureometer.'

RESULTS

The following two points must be taken into consideration when studying the figures that follow :

- (I) Amongst the cases examined in England the heading 'Malaria. Parasites present,' means that parasites were actually present in the blood at the time that the urine was passed. In the cases examined in West Africa, as it was not always possible to examine the urine on the same day as the positive blood film was obtained, all that can be said with certainty is that parasites were present within a few days previous to the urinary examination.
- (2) No distinction between the different species of malaria parasites is made in the tables ; actually when compiling the figures the different urinary findings accompanying malignant tertian, benign tertian, or quartan malaria were recorded, but as the differences found were of a trivial character no record of them has been retained in the paper.

TABLE I.

Showing the frequency of urobilinogen in the urine of 200 individuals in England and Sierra Leone.

1	Engi	AND					Fre	etown, S	ierra Leo	ONE		
	Europ	beans				English	residents			Nati	ves	
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present
Number of cases examined	. 18	30	4	19	18	IO		28	33	24	8	8
Percentage positive		30	50	84	17	0		39	6	33	62	87

Remarks. In none of the cases recorded above was urobilinogen detected without urobilin also being found.

TABLE II.

Showing the frequency of urobilin in the urine of 200 individuals in England and Sierra Leone.

		Eng	LAND					Free	town, Sii	erra Leon	ЧE		
		Euro	opeans				English r	esidents			Nati	ves	
	Nor	mal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present
Number of cases examined .	I	8	30	4	19	18	10		28	33	24	8	8
Percentage positive .	I	I	53	75	95	39	IO		89	12	50	87	87

Remarks. Under the heading 'Mixed hospital cases' there are included four cases of amoebic dysentery; one of these gave a positive urobilin reaction. A case which had been operated on for amoebic liver abscess twelve months previously and which still showed signs of hepatitis was also tested, but gave a negative reaction. In connection with this it may be noted that Grall, as quoted by the *Annals of the Clin. Laboratories* (1924), 'Considers that in a colonial subject abundant urobilinuria ought forthwith to make one suspicious of amoebic hepatitis.'

TABLE III.

Showing the frequency of albumin in the urines of 202 individuals in England and Sierra Leone.

	En	GLAND					Free	town, Sii	erra Leon	1E		
	Eur	opeans				English r	esidents			Nat	ives	
	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present			
Number of cases examined	18	30	4	21	18	IO		28	33	24	8	8
Percentage positive	0	37	75	. 71	-5	20		63	.24	42	62	36

Remarks. The large number of albuminurias among normal natives in Sierra Leone is probably explained by the fact that the individuals examined were convicts, many of whom were known to be suffering from chronic gonorrhoea.

Та	BLE	IV.	

Showing the frequency of casts in the urine of 200 individuals in England and Sierra Leone.

	Eng	LAND					Free	town, Sie	rra Leon	E		-
	Euro	peans				English r	esidents			Nati	ives	
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present
Number of cases examined	18	30	4	19	18	IO		28	33	24	8	8
Percentage positive	0	17	25	53	0	. 10	•••	25	3	29	12	12

Remarks. The low percentage of malaria cases showing casts amongst the people examined in West Africa, as compared with those in England, may be due to their having received early and efficient quinine treatment, while the cases examined in England had suffered from a lack of thorough treatment during a long sea voyage.

TABLE V.

Showing the frequency of indican in the urine of 202 individuals in England and Sierra Leone.

	Eng	LAND					Free	town, Si	erra Leoi	NE		
	Euro	peans				English 1	residents			Nati	ves	
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites Present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites Present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites Present
Number of cases examined	19	- 29	4	21	18	10		28	33	24	8	8
Percentage positive	21	41	0	43	II	IO	• • • •	25	6	21	0	12

Remarks. Judged on these figures indicanuria would appear to be less frequent in the tropics than in England; for further European figures see Gordon (1923b).

TABLE VI.

Showing the frequency of bile pigments in the urine of 200 individuals in England and Sierra Leone.

	Enc	GLAND					Free	TOWN, SI	erra Leo	NE		
	Euro	peans				English	residents			Nat	ives	
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites Present	Normal	Mixed hospital case s	Clinical malaria	Malaria. Parasites Present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Para ites Present
Number of cases examined	18	30	4	19	18	10	•••	28	33	24	8	8
Percentage positive	0	7	0	0	0	0		O	0	, O	0	0

Remarks. From this it will be seen that bile pigments, were only detected in the urine of two out of 200 individuals examined; in both these cases the patient was suffering from well-marked obstructive jaundice.

TABLE VII.

Showing the frequency of urea above three per cent. in the urines of 57 individuals in England.

		Engl	AND	
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present
Number of cases examined	15	22 .	4	· 16
Percentage positive	7	9	0	56

TABLE VIII.

Showing the results of urinary examinations in three cases of general paralysis of the insane, before, during, and after an attack of simple tertian malaria. (Note—' + ' signifies presence of, ' \circ ' absence of, and ' — ' no examination made.)

				JANUARY 9 10 11 12 13 14 16 18 19 21 23 25 27 28 29																F	EBRU	AR¥	
Date, 1924		8	9	IO	II	12	13	14	16	18	19	21	23	25	27	28	29	31	2	4	6	8	10 and 12
First temperatu	re	+		•••	••••						••••									••••			
Parasites		0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0 30=0	0	0	0	0	0.22
Quinine Grains	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0
Urobilinogen		0	0	+	+	+	+	0	0	+	+	+	+	+	+		+	0	+	0	0	0	0
Urobilin		0	0	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	0	0	0	0
Albumin		0	0	0	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	0	0	0
Casts		0	0	0	0	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	0	0
Indican		0	+	+	+	+	+	+	+	0	+	+	+	0	0	-	+	. 0	0	+	0	+	+
Bile pigments		0	Ö	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0
Urea above 3%		-	-	0	+	+	+	+	+	+	+	+	0	0	0	-	0	0	0	0	0	0	0

CASE 1. Anophelines infected with P. vivax fed on patient, 31.12.23.

TABLE VIII (cont.).

CASE	2.	Anophelines	infected	with	<i>P</i> .	vivax	fed	on	patient,	21.2.24	1.
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			Fe	BRUAI	RY								Mare	сн			,					April
Date, 1924	•••	••••	21	24	27	· I	4	6	8	10	12	14	15	16	18	20	22	24	26	28	30	13
irst temperature		•••						+								•••						
Parasites			0	0	.0	0	0 5=0	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
Quinine grains 30			0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0
Jrobilinogen		• • •	0	0	0	0	0	0	0	0	0	+	-	+	+	0	0	0	0	0	0	0
Jrobilin			0	0	0	0	0	+	0	0	+	+	-	+	+	+	0	0	0	0	0	0
Albumin			0	0	0	0	0	0	0	+	+	+		+	+	+	+	0	0	0	0	0
Casts			0	0	0	0	0	0	+	+	+	+		+	+	+	+	0	0	0	0	0
ndican		•••	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0
Bile pigments			0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0
Jrea above 3%			0	0	0	0	0	0	+	+	0	0	-	+	0	0	0	0	0	0	0	0

TABLE VIII (cont.).

CASE 3. Anophelines infected with *P. vivax* fed on patient, 22.2.24. Note—Slight nephritis was present before infection.

			F	BRUA	RY								M	ARCH								Арі	RIL
Date, 1924		• •••	22	25	28	2	5	7	8	9	10	12	14	16	18	20	22	24	26	28	30	10	18
First temperatur	e	•••			•••			+	•••	••••					••••		•••	•••					
Parasites			0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+	0	0	0
Quinine grains 1	D		0	0	0	0	0	0	0	Ó	0	0	0	0	0	0	0	0	0	+	+	+	0
Urobilinogen			0	0	0	0	0	-	+		+	+	0	0	+	+	+	+	+	+	+	0	0
Urobilin			0	0	0	0	0		+	_	+	+	+	+	+	+	+	+	+	+	+	0	0
Albumin			Tr.	Tr.	Tr.	Tr.	Tr.	-	+		+	+	+	+	+	+-	+	Tr.	Tr.	Τr.	Tr.	0	0
Casts			+	0	0	+	0	_	+		+	+	0	+	+	o	0	0	0	0	0	0	0
Indican			+	0	0	+	0		+	-	0	0	0	0	0	0	0	0	0	0	0	0	0
Bile pigments			0	0	0	0	0		0	-	0	0	0	0	0	0	0	0	0	0	0	0	0
Urea above 3%			+	0	0	0	0		0	-	0	0	0	+	0	0	0	0	0	0	0	0	0

Remarks. In Cases I and 2 urobilinogen and urobilin first appeared either on the same day, or else later than, the parasites ; in Case 3 both urobilinogen and urobilin preceded the parasites by two days, in all three cases these substances persisted for one to five days after the blood had become negative. Albumin and casts appeared in Cases I and 2—and were increased in Case 3—within three days of the first positive blood finding, and persisted in Cases I and 2 for about a week after the disappearance of parasites; in Case 3 it will be noted that casts ceased to appear in the urine for some days prior to the blood becoming negative.

The number of days during which urobilinogen, urobilin, albumin and casts persisted in the urine after parasites had disappeared may also be noted in the following additional cases. *Case A*. Urobilinogen less than four days, urobilin more than four days, albumin more than four days, casts less than four days. *Case B*. Urobilinogen less than four days, urobilin less than four days, albumin more than four days, casts less than four days. *Case C*. Urobilinogen, urobilin, and albumin less than ten days, casts more than ten days. *Case D*. Urobilinogen and urobilin less than seven days, albumin or casts did not appear. *Case E*. Urobilinogen and urobilin more than nine days, albumin and casts less than nine days.

T	AB	LE	IX.	

		JANUARY			February																	
Date, 1924						29	30	31	I	2	3	4	5	6	7	8	9	10	II	12	17	18
Temperature						98	100	101	102	102	103	98	98	98	98	98	98	98	98	98	101	10
Urobilinogen							0	-	+	-	-	0		0		+		+	-	0	-	+
Urobilin			•••	• • •		-	+	-	+ .	-	-	0	·	0		+		+		0		+
Albumin					•••	-	0		0		-	0	-	0	.—	0	_	0	_	0		ó
Casts				•••	••••		0	-	0	-		0		0	_	0	_	0	-	0		0
Indican		•••		•••		_	0	-	+			0	-	+		+		+		0		0
Bile pigments	• •,• ;	•••			•••	_	0		0	_	-	0		0		0		0		0		0
Urea above 3%			•••	•••	•••		0	-	0			0	-	0		0		0	-	0	-	0

Showing the results of urinary examinations during pyrexial and apyrexial periods in a case of lymphadenoma. Note-The patient had never been abroad.

Remarks. Note the irregularity of the appearance of both the urobilinogen and the urobilin and that they do not always correspond to the pyrexial periods.

TABLE X.

Showing the frequency of a combination of urobilin, and albumin (with or without urobilinogen, casts, indican, bile pigments, or raised urea percentage), in the urine of 200 individuals in England and Sierra Leone.

	Eng	LAND			Freetown, Sierra Leone										
Europeans						English r	esidents		Natives						
	Normal	nal Mixed Clinical M hospital malaria Pa cases pr		Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present			
Number of cases examined	18	30	4	19	18	IO		28	33	24	8	8			
Percentage positive	0	13	50	84	0	0		46	0	25	62	37			

CONCLUSIONS

As regards positive findings, it can at once be stated that no individual test was found to have any real value as an aid in the diagnosis of malaria, one and all occurring with considerable frequency not only in diseases other than malaria but also in the normal individual, as is clearly shown in Tables I to VII; so far, therefore, as any single test is concerned the results obtained are in accordance with the views expressed by Lane (1923) who remarks

'There emerges from all this the conclusion, old but needing as much emphasis as ever it did. In any particular case the finding of parasites is the only justification for a positive diagnosis of present malarial infection; but the failure to find them by the ordinary techniques cannot justify a confident diagnosis that malaria is certainly absent.'

If, however, the figures furnished in the various tables are consulted, more especially Table X and the three cases recorded in Table VIII, it will be seen that a combination of urobilin and albumin in the urine, although no certain proof, is at least strong evidence in favour of malarial infection; whether such evidence is strong enough to warrant immediate treatment with quinine is a matter which can only be decided by the exigencies of the case and the opinion of the physician. As regards negative findings, the absence of urobilin (which implies the absence of urobilinogen; see remarks Table I) from the urine of a suspected malaria case appears to be of very considerable value, for not less than 87 per cent. of all true cases of malaria, whether examined in England or West Africa, showed the presence of urobilin and it has been shown (Table VIII) that urobilin is sometimes a precursor of the malaria parasites and usually persists after the latter's disappearance. It is, therefore, probably legitimate to consider that a case which has had a rise of temperature within the past forty-eight hours and does not exhibit urobilin in the urine is extremely unlikely to be suffering from malaria.

REFERENCES

- ACTON, H. W., and KNOWLES, R. (1913). The diagnosis of latent malaria. Indian Journal of Medical Research, Vol. I, No. 1, p. 167.
- BALLERSTEDT (1924). Das Verhalten des Urobilins bei Malaria. Arcb. f. Schiffs-u-Trop.-Hyg., Vol. XXVIII, Nos. 3 and 4, p. 100.
- COLE, S. W. (1920). Practical Physiological Chemistry, 6th Ed., p.37.
- DI PACE, I. (1923). A proposito dell'urobilinuria nella diagnosi della malaria latente. *Policlinico*, Vol. XXX, No. 28, p. 894.
- GORDON, R. M. (1923a). The Urine in Malaria. Ann. Trop. Med. & Parasit., Vol. XVII, No. 4, p. 556.
- ----- (1923b). The frequency of indicanuria. Ann. Trop. Med. & Parasit., Vol. XVII, No. 4, p. 549.
- GRAHAM, W. M. (1911). An investigation of the effects produced upon the excretion of urinary pigments by salts of quinine. Ann. Trop. Med. & Parasit., Vol. V, No. 3, p.391.
- GRALL (1924). As quoted by Annals of the Clin. Laboratories, Vol. XIII, No. 1, p. 19.
- LANE, C. (1923). The malarias. Recent studies and their bearings. Trop. Dis. Bull., Vol. XX, No. 10, p. 773.
- LEVY, MOISE D. (1923). Malarial nephritis. Southern Medical Journal, Vol. XVI, No. 12, p. 942.
- MACCORMAC, H., and DODDS, E. C. (1923). An investigation into the effects of the arsenobenzol treatment of syphilis on liver function. Brit. Med. Jl., Vol. ii, p. 1201.
- PLEHN, A. (1908). The diagnosis of latent malaria. Brit. Med. Jl., Vol. ii, p. 1357.
- SIMPSON, G. C. E. (1910). On haemoglobin metabolism in malarial fever. Ann. Trop. Med. and Parasit., Vol. IV, No. 3, p. 313.
- SORENSEN, N. (1914). Die Urobilin Sekretion im Harne bei Malaria, besonders beim Schwarzwasserfieber. Arcb. f. Schiffs-u-Trop. Hyg., Vol. XVIII, No. 5, p. 159.
- SURVEYOR, N. F. (1910). Some observations on malaria in relation to splenic enlargement and the treatment of the crescentic stage. Ann .Trop. Med. & Parasit., Vol. IV, No. 3, p. 333.